SUMMARY

The observation that both anticholinergics and dopamine agonists ameliorate parkinsonian symptoms has generated a great number of neurochemical studies aimed to establish an interaction between these two transmitter systems. From these studies it was concluded that dopamine inhibits the output of acetylcholine in the striatum via a dopamine D2 receptor. Furthermore, the increases in the output of acetylcholine or the decreases in the tissue content of acetylcholine after systemic administration of dopamine receptor blockers indicated that dopamine *tonically* inhibited striatal cholinergic neurons. Before the development of a brain microdialysis method for the measurement of acetylcholine, the studies on the dopamine-acetylcholine interactions in the striatum were done using in vitro release studies, tissue measurements or using push-pull cannulas in gallamine immobilized cats. Since brain microdialysis provides a unique opportunity to measure neurochemical events in freely moving, conscious animals, the striatal dopamine-acetylcholine interactions were investigated using this method.

Initially we failed to show an interaction between nigrostriatal dopaminergic fibers and cholinergic interneurons using brain microdialysis. Since these findings were largely at variance with the prevailing view in the scientific community, we decided to study some methodological aspects pertaining to microdialysis studies to validate the method and to provide further evidence that would either confirm or deny the existence of a striatal dopamine-acetylcholine interaction.

In the **introduction** the role of acetylcholine and dopamine in basal ganglia functioning is discussed. Both dopamine and acetylcholine are thought to be important in the gating of information that is largely derived from the cortex flowing through the striatal cell mosaic to the basal ganglia output centers. Furthermore, the possibility that dopamine and acetylcholine interactions occur via a common target neuron is discussed in the light of electrophysiological and anatomical evidence. It is concluded that there might be a convergence of cholinergic and dopaminergic input on the GABA-ergic medium spiny neuron, but

that electrophysiological data not clearly show an antagonistic interaction of the transmitters dopamine and acetylcholine.

In **chapter 1** we discuss the effect of neostigmine (a acetylcholinesterase inhibitor), coinfused in our artificial CSF, on muscarinic autoreceptor responses. It is shown that 0.1 μmol/l neostigmine increases the extracellular levels of acetylcholine about ten-fold and that the increase in extracellular acetylcholine results in the attenuation of the effect of the muscarinic agonist oxotremorine and a potentiation of the effect of the muscarinic antagonist atropine.

In **chapter 2** the muscarinic autoreceptor that modulates the output of acetylcholine from the striatum is further characterized by infusing the selective muscarinic antagonists 4-DAMP, AF-DX 116 and pirenzepine. It is shown that 4-DAMP increases the output of acetylcholine from the striatum most potently, followed by pirenzepine and AF-DX 116 respectively. From these observations we conclude that an M_3-muscarinic autoreceptor modulates the overflow of acetylcholine from the striatum.

In **chapter 3** the effect of the calcium concentration of the microdialysis perfusion fluid is tested both on autoreceptor and heteroreceptor responses of cholinergic and dopaminergic neurons. Whereas the output of acetylcholine was unresponsive to D2 receptor activation at elevated perfusion fluid calcium concentrations (3.4 mmol/l), at more physiological calcium concentrations (1.2 mmol/l) an inhibition of the output of acetylcholine could be measured after D2 receptor stimulation. It is shown that the attenuation of the effect of dopamine agonists can be attributed to muscarinic autoreceptor occupation at 3.4 mmol/l calcium. Infusion of the dopamine receptor antagonist (-)-sulpiride did not affect the output of acetylcholine either at 1.2 mmol/l or 3.4 mmol/l calcium. The muscarinic agonist oxotremorine also decreased the output of acetylcholine at 1.2 mmol/l calcium but not at 3.4 mmol/l calcium. The output of striatal dopamine could be increased after infusion of the dopamine antagonist (-)-sulpiride and decreased after infusion of the dopamine agonist (-)-N-0437 both at 1.2 mmol/l and 3.4 mmol/l calcium present in the perfusion fluid. Neither oxotremorine, nor atropine could affect the output of striatal dopamine at both calcium

concentrations. The percentual increase in the output of dopamine after infusion of the dopamine releasing drug (+)-amphetamine or the dopamine uptake inhibitor nomifensine was greater at 1.2 mmol/l than at 3.4 mmol/l calcium.
These studies indicate that the output of acetylcholine is modulated by muscarinic autoreceptors and dopaminergic (D2) heteroreceptors. The D2 receptors are probably not tonically activated. The output of striatal dopamine is modulated by dopamine (D2) autoreceptors but not by muscarinic heteroreceptors.

Whereas in chapter 3 the drugs were infused into the striatum, in **chapter 4** the results of systemic applications of dopamine agonists and antagonists are studied on the release of acetylcholine. The dopamine agonists apomorphine and N-0437 decrease the output of striatal acetylcholine, whereas the dopamine antagonists sulpiride and haloperidol increase the output of striatal acetylcholine.

In **chapter 5** the effects of dopamine D1 and D2 receptor ligands, the dopamine releaser (+)-amphetamine and the dopamine uptake inhibitor nomifensine are studied on the release of striatal acetylcholine as a function of the post-implantation interval of a microdialysis probe. It is shown that striatal dopamine D2 but not D1 receptors are involved in the modulation of striatal acetylcholine release. The dopamine D2 receptors are not occupied 16-24 h after probe implantation, but a tonic interaction between dopaminergic and cholinergic systems can be seen 40-48 h after probe implantation. The dopamine releasing drug (+)-amphetamine and the dopamine uptake inhibitor nomifensine dose-dependently increase the output of striatal dopamine. However, only when the extracellular dopamine levels are more than sevenfold increased, the output of striatal acetylcholine is affected. These studies confirm the existence of a dopamine D2 receptor mediated inhibition of the release of acetylcholine and the existence of a tonic inhibition of the cholinergic interneuron in the striatum by dopamine. Furthermore, the importance of the post-implantation of a microdialysis probe for the pharmacological responsiveness of striatal cholinergic neurons is shown.
The theme of **chapter 6** might be clinically relevant. Patients with Parkinson's disease are currently treated with L-DOPA, an ergot drug of a combination of these drugs. The neurochemical effect of the ergot drugs and L-DOPA on the release of

striatal acetylcholine was assumed to be similar. However, in this study we show that the output of acetylcholine from the striatum is increased after systemic application of L-DOPA. In contrast, the output of striatal acetylcholine decreases after systemic injections of bromocriptine. These effects are not only seen in animals with an intact nigrostriatal dopaminergic system, but also in animals treated with the dopaminergic neurotoxin 6-hydroxydopamine. It is speculated that the increased output of striatal acetylcholine is the result of the stimulation of extrastriatal dopamine D1 receptors. This topic is further discussed in the **discussion**.

Not only dopaminergic systems modulate the output of acetylcholine from the striatum. In **chapter 7** we show that in addition GABA-ergic receptor activation by direct acting receptor ligands modulates the output of striatal acetylcholine. Striatal acetylcholine release may be tonically inhibited via a $GABA_a$-receptor. Since intrastriatal infusion of dopamine D1 agonists that increase the output of GABA does not affect the output of acetylcholine, we conclude that the GABA-ergic modulation of striatal cholinergic neurons occurs via GABA-ergic cell elements in the striatum that have no D1 receptors.